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Winter and spring night-warming improve root extension and soil nitrogen supply to increase nitrogen uptake and utilization of winter wheat (*Triticum aestivum* L.)

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ABSTRACT

Elucidating the effects of asymmetric warming during winter and spring will help develop a feasible crop management strategy for climate change. Field experiments were conducted using the Yangmai-13 (vernal type) and Yannong-19 (semi-winter type) winter wheat cultivars to investigate the effects of night-warming during winter (warming by 1.47–1.53 °C from tillering to jointing), spring (warming by 1.68–1.77 °C from jointing to booting), and winter + spring (warming by 1.53–1.60 °C from tillering to booting) on plant growth and N utilization in 2014–2016. The results showed that the grain yield, N agronomic efficiency (NAE), and N recovery efficiency (NRE) of both cultivars were highly increased in response to night-warming, which were associated with enhanced dry matter and N accumulation, and winter + spring night-warming resulted in greater increases than winter night-warming and spring night-warming. Furthermore, the increase in pre-anthesis N accumulation was much higher than after anthesis, resulting in a greater increase in post-anthesis dry matter accumulation due to more leaf N distribution at anthesis to support photosynthetic production. Root growth characteristics (i.e., root length, surface area and volume, and root bleeding intensity) were significantly promoted, which favored plant N uptake. Soil urease and protease activity as well as the net N mineralization rate, which are involved in soil N supply capacity, were increased, whereas soil inorganic N content and apparent N surplus were clearly decreased, which indicated that plant N uptake capacity was highly improved in response to night-warming conditions. In conclusion, winter and spring night-warming improve pre-anthesis root growth and N uptake ability to promote plant growth, resulting in increased N utilization efficiency with reduced N fertilizer loss, and winter + spring night-warming has more advantages for N uptake and utilization of winter wheat.

1. Introduction

Warming is a main impact of global climate change. In the past 100 years, the global air temperature has increased by approximately 0.65–1.06 °C, and it is likely to increase by another 0.3–4.8 °C by the end of this century (IPCC, 2014). Global warming causes diurnal warming asymmetry, as the amplitude of the temperature increases in winter and spring are greater than those in summer and autumn, and warming is greater at night than during the day (Lobell, 2007; Zhou et al., 2007; Peng et al., 2013; Suonan et al., 2017). Temperature is a key factor regulating crop development and growth (Lobell et al., 2011). Understanding the impact of asymmetric warming on crop production may facilitate the development of food security strategies that help adapt agriculture to future climate changes.

Numerous efforts have been made to understand the impacts of warming on wheat production. Some studies have suggested that climate warming will decrease wheat yield because warming shortens the length of the wheat growth period, resulting in large declines in biomass production (Lobell and Asner, 2003; Hussain and Mudasser, 2007; You et al., 2009). Others have reported that the production of wheat might benefit from warming because warming increases the number of grains per ear and 1000-grains weight or primary biomass production by stimulating net photosynthesis (Tian et al., 2012; Fan et al., 2015; Zheng et al., 2017). These observations suggest that wheat yield does not always respond consistently to warming.

Nitrogen is the most essential nutrient for wheat growth,

Abbreviations: N, nitrogen; NUE, N use efficiency; NRE, N recovery efficiency; NAE, N agronomic efficiency; NR, nitrate reductase; GS, glutamine synthetase

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productivity and grain quality among all nutrients (Fageria and Baligar, 2005). Improved wheat N use efficiency (NUE) would be of economic benefit to farmers and would help reduce environmental contamination associated with excessive inputs of N fertilizer (Stevens et al., 2005; Delin et al., 2008). Root growth is an essential parameter for plant N uptake, and improved root growth helps increase plant N uptake and reduce nitrate leaching loss to the environment (Foulkes et al., 2009; Rasmussen et al., 2015). A few studies have investigated the effects of warming on plant root morphology and activity (Björk et al., 2007; Bai et al., 2010; Yin et al., 2012). For example, Yin et al. (2013) reported that experimental warming increases root length, root biomass, and the root/shoot mass ratio in two coniferous species. Tian et al. (2014) reported that experimental warming increases root dry biomass and root activity of winter wheat at anthesis, resulting in improved plant N uptake. However, most of these studies focused on the entire plant growth period, and little is known about variations in root morphology in the soil profile and root activity of winter wheat in terms of asymmetric warming.

Soil inorganic N is easily absorbed by plants and represents the soil N supply capacity (Liu et al., 2003). Many studies have revealed that warming increases the soil net N mineralization rate, resulting in increased inorganic N content in soil, which helps improve plant N uptake (Butler et al., 2012; Bai et al., 2013; Zhang et al., 2015). Furthermore, soil enzymes play vital roles in the decomposition of soil organic matter and soil nutrient mineralization, and temperature is an important factor determining soil enzyme activity (Gong et al., 2015). Therefore, climate warming is likely to significantly affect soil enzyme activity and consequently alter soil inorganic N content, which will likely change plant N uptake (Baldrian et al., 2013). The most important enzymes involved in soil N cycling are proteases and ureases (Sardans et al., 2008a). Proteases are involved in the first step of N release by hydrolysing the peptide bonds between amino acids as an indispensable step for N uptake by plants. Ureases hydrolyse urea to ammonium and carbon dioxide, and ammonium can be directly absorbed and utilized by plants. Several studies have shown that warming increases soil urease and protease activity because warming increases microbial activity, thus increasing the release of enzymes (Wallenstein et al., 2010; Xu et al., 2010; Liu et al., 2014). Others have reported that warming decreases soil urease and protease activity because warming reduces soil water content, and limited soil water can directly affect enzyme activity (Brzostek et al., 2012; Zhao et al., 2014; Davidson et al., 2015). However, most of these studies were carried out in grassland or forest ecosystems; very few studies are available on the effects of asymmetric warming on the net N mineralization rate and enzyme activity involved in soil N cycling in wheat fields. Wheat mainly grows during the winter and spring seasons, when more warming is anticipated. Therefore, it would be of great practical significance to study the change in NUE of wheat in response to winter and spring night warming.

The objectives of this study were to (1) evaluate the effects of winter and spring night-warming on N uptake and utilization of winter wheat and (2) identify how winter and spring night-warming affect root growth and soil N supply and their relationship to wheat N uptake and utilization. The results are intended to provide a theoretical basis for improving the NUE of wheat under future climate change.

2. Materials and methods

2.1. Experimental design

Field experiments with two wheat cultivars Yangmai-13 (vernal type) and Yannong-19 (semi-winter type) were conducted from 2014 to 2016 in Nanjing (32°04′N, 118°76′E), China. Weather conditions and the initial status of the 0–60 cm soil layer of the experimental site during the wheat growing season are given in Fig. 1 and Table 1 respectively.

The experiment consisted of macro-plot experiment and micro-plot experiment. The macro-plot experiment comprised of a randomized complete block with three replications and four warming treatments, including winter night-warming treatment (WW), spring night-warming treatment (SW), winter + spring night-warming treatment (WSW) and no warming control (NW). The WW, SW and WSW were applied from tillering to jointing (from Dec. 20, 2014 to Mar. 5, 2015 and from Dec. 23, 2015 to Mar. 4, 2016), jointing to booting (from Mar. 6, 2015 to Apr. 6, 2015 and from Mar. 5, 2016 to Apr. 4, 2016), and tillering to booting (from Dec. 20, 2014 to Apr. 6, 2015 and from Dec. 23, 2015 to Apr. 4, 2016), respectively.

Based on the technique of passive night warming (PNW) (Beier et al., 2004; Fan et al., 2015), a removable plastic membrane of manual control was constructed. The warmed plots were covered with the plastic membrane from sunset to sunrise (from around 19:00 to 07:00 on next day). To ensure normal respiration of the crops at night, the plots were provided with proper ventilation. To keep each plot with the same precipitation, the plastic membranes were rolled up at night when it rained or snowed. The warming facility was 3 m × 5 m in area and 2 m in height. Canopy temperatures were automatically recorded using a dual-channel LCD temperature instrument (NZ-LBR-F11, Nanjing Nengzhao Electronic Instrument Co., Ltd., China) after every 10 min interval, whereas, temperatures in 5 cm soil layer were automatically recorded by using a digital data logger (EM 50, Decagon Devices, Inc., USA) at every 10 min interval. The increase in the mean night temperature of canopy and 5 cm soil layer between treatments and the control are given in Fig. 2.

The plot size was 2 m × 4 m and seeding density was 2.25×10^6 seeds ha^{-1} with a spacing of 0.25 m between rows. All plots were supplied with 240 kg N ha^{-1}, 105 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1}, in the form of urea, superphosphate and potassium chloride fertilizers, respectively. 120 kg ha^{-1} N and total P_2O_5 and K_2O were applied before sowing. The remaining N was applied in splits at jointing and booting stages, with 60 kg N ha^{-1} at each stage. Sowing dates were 27 October in 2014 and 4 November in 2015. Fungicides and pesticides were applied at jointing, booting and 10 days after anthesis to control diseases and pests. The precipitation was ample for winter wheat growth, thus, no irrigation was applied during the period of experiments.

The micro-plot experiment was designed same as the macro-plot experiment using Yangmai-13 in 2015–2016. The micro-plots were set within macro-plots by polyvinyl chloride (PVC) tubes with 25 cm diameter and 105 cm height to monitor the root morphology, this method was also used by (Giacomini et al., 2010; Shi et al., 2012a). To keep the micro plots with soil conditions similar to field experiments, soil was dug out and separated into four layers: 0–20, 20–40, 40–60 and 60–100 cm. Soil layer of 20–100 cm was backfilled into the PVC tube in the correct order, followed by watering to consolidate the layers. After that, the PVC tubes were buried into macro-plot with the top edge at 5 cm above the ground. Soil layer of 0–20 cm was backfilled into the PVC tube after mixing basal fertilizer. All micro-plots received same N rate with field experiment.

2.2. Plant and soil sampling and analysis

Three replications of each treatment were sampled at sowing (0 days after sowing, 0 DAS), tillering (44 DAS), overwintering (80 DAS), regreening (112 DAS), jointing (125 DAS, before topdressing), booting (154 DAS), anthesis (168 DAS), filling (182 DAS, 14 days after anthesis) and maturity (206 DAS). Plant shoots were separated into leaves, culms, chaffs, and grains. Fresh samples were first put into the oven at 105 °C for 30 min to deactivate enzymes, and then dried at 70 °C till a constant weight reached for dry weight determination. The dried samples were milled. The total N content of the plant samples was determined using the semi-micro Kjeldahl method (Santos and Boiteux, 2013). Two 2 m long rows (1 m^2) of plants were marked at anthesis in the center of the plots to measure grain yield at maturity.

Soil samples to a depth of 60 cm were separated into three layers:

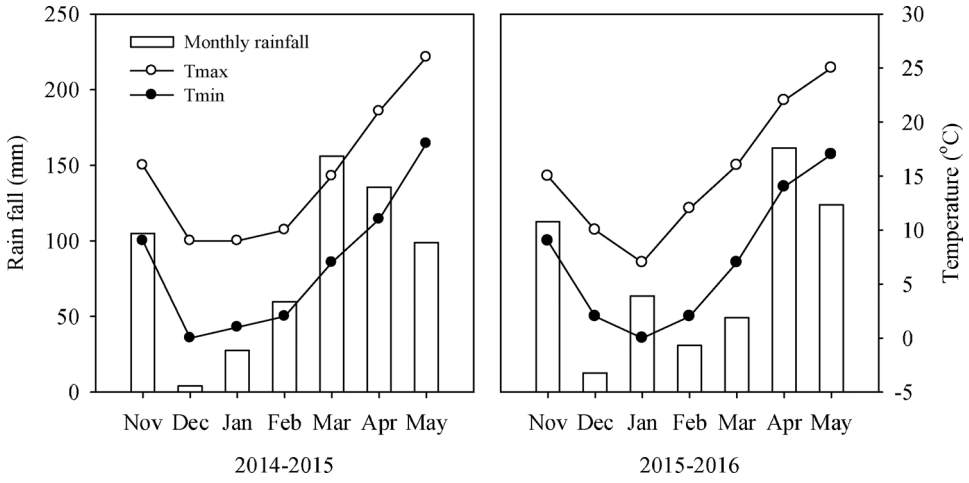

Fig. 1. Monthly mean rainfall and mean maximum and minimum temperatures during the wheat growing season from 2014 to 2016 in Nanjing, China.

0–20, 20–40, and 40–60 cm. Fresh soil samples were thoroughly mixed and representative sub-samples were extracted immediately using 2 M KCl solution (soil solution ratio: 1:5) by shaking for 1 h on a rotary shaker (180 rev min^{-1}), followed by filtration. The extracts were directly analyzed for the content of NO_3^--N and NH_4^+-N using an automated continuous flow analyzer (Seal Autoanalyzer 3, Seal Analytical, Inc., Germany) or stored at 4 °C in a freezer until analysis by the same method within 1 week. Soil bulk density was determined by the cutting ring method. Soil water content was determined by drying the soil samples in an oven at 105 °C until a constant weight.

Soil net N mineralization rates were measured from in situ incubations using the buried bag technique (Adams et al., 1989). The incubations were performed using perforated PVC tubes (20 cm in height and 6 cm in diameter). Parafilm covered the top of each tube to avoid leaching of nitrate. The technique prevents plant uptake of mineralized nutrients but allows uptake by the microorganisms. The soil samples were analyzed for NO_3^--N and NH_4^+-N as the initial sample for measurement of net mineralization. The soil samples in the buried bags were retrieved after 30 days of incubation (at filling stage we retrieved them after 15 days) and analyzed as the final sample. The difference between the initial and final inorganic N concentration (NO_3^--N plus NH_4^+-N) was used to calculate net N mineralization rate.

Soil enzymatic activities were measured using the following methods described by Guan et al. (1984). Urease activity was analyzed with pH 6.7 citrate acid buffer solution. We added 1 ml of toluene to 5 g of soil in a wide-mouthed flask (50 ml). After 15 min, 10 ml of 10% urea and 20 ml of citrate acid buffer solution (pH 6.7) were added to the flask and then incubated this mixture for 24 h at 37 °C. The suspension was filtered through qualitative filter-slow paper and 3 ml of the filtrate was extracted to a volumetric flask (50 ml). After adding 20 ml of distilled water, 4 ml of 1.3 M sodium phenoxide solution and 3 ml of sodium hypochlorite solution (active chlorine 0.9%) successively, the absorbance of the liberated NH_3-N in the filtrate was measured at

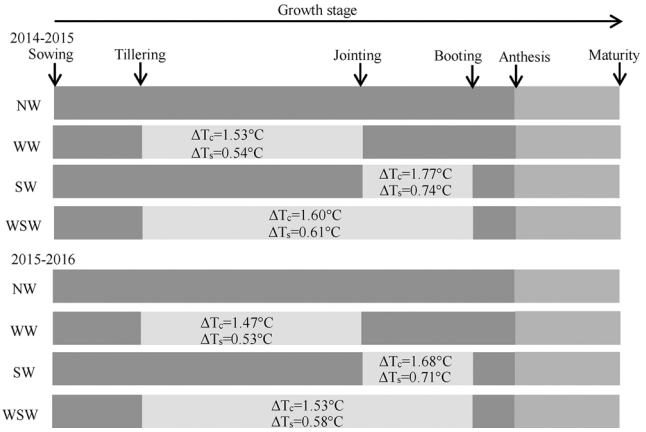

Fig. 2. Schematic representation of experimental design and treatments. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. ΔT_c and ΔT_s refer to the increase in the mean night temperature of canopy and 5 cm soil layer between treatments and the control, respectively. Mean night temperature is the mean in all temperature data on a 10-min interval from 19:00 to 07:00 h.

578 nm. Urease activity was expressed as mg NH_3-N g^{-1} soil 24 h^{-1}.

Protease activity was analyzed with casein solution (pH 7.4). We added 1 ml of toluene to 5 g of soil in a wide-mouthed flask (50 ml). After 15 min, 10 ml 1% casein solution were added to the flask and then incubated this mixture for 24 h at 30 °C. The suspension was filtered through qualitative filter-slow paper and 5 ml of the filtrate was extracted to a test tube (50 ml). Afterwards, 0.5 ml 0.05 M sulfuric acid and 3 ml 20% sodium sulfate were added to the filtrate, centrifuged for 15 min at 6000 rpm and then the solution was extracted to a volumetric flask (50 ml). After adding 1 ml 2% ninhydrin solution, the volumetric flask was heated for 10 min in a 100 °C electric-heated thermostatic

Table 1
Soil properties of experimental site at the beginning of experiment in two growth seasons.

Growing season	Soil layer	Organic matter	Total N	Olsen-P	Exchangeable	Nitrate N	Ammonium N
	(cm)	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	K (mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
2014–2015	0–20	11.71	1.25	9.75	70.39	7.72	3.64
	20–40	10.63	1.13	8.34	65.43	4.24	2.86
	40–60	8.74	0.97	7.67	60.17	2.32	2.43
2015–2016	0 – 20	10.95	1.33	9.85	74.30	8.54	3.43
	20–40	10.45	1.21	8.28	68.39	5.33	2.68
	40–60	9.33	1.04	7.57	63.70	2.71	2.33

water bath and then cooled for 3 min in cold water. The absorbance of the liberated amino acid in the filtrate was measured at 560 nm. Protease activity was expressed as mg glycine g^{-1} soil 24 h^{-1}.

Fully expanded fresh leaves at jointing, booting, anthesis, and filling stages were sampled to analyze the changes in enzyme activities of N metabolism. Nitrate reductase (NR) activity was determined according to Abd-El Baki et al. (2000). Extracts were prepared from leaf samples harvested 3 h after dawn, when NR activity was close to the maximum value (Man et al., 1999). Frozen leaves (0.1 g) were ground using a mortar and pestle in liquid N. One milliliter of extraction buffer (100 mM HEPES-KOH, pH 7.6, 20 mM $MgCl_2$, 5 mM dithiothreitol [DTT], 10 uM flavin adenine dinucleotide phosphate, 10 uM leupeptin, 0.2 mM phenylmethyl-sulfonyl fluoride, 1 mM Pefabloc, 0.6% polyvinylpolypyrrolidone, and 0.05% casein) was added to the still-frozen powder. Then the suspension was centrifuged for 12 min (4 °C; 16000 × *g*) and the supernatant was removed and maintained at 4 °C. The reaction medium consisted of 50 mM HEPES-KOH, pH 7.6, 5 mM KNO_3, 0.2 mM NADH, 10 uM flavin adenine dinucleotide phosphate, 1 mM DTT, and either 20 mM $MgCl_2$ or 20 mM ethylene diaminete tracetic acid (EDTA) in a total volume of 1000 uL. The reaction was started by adding 140 uL extract and terminated after 5 min by adding 128 uL zinc acetate solution (500 mM). After a short centrifugation (4 °C, 5 min; 16000 × *g*), 8 uL phenac in metasulfate was added to 1000 uL supernatant to oxidize excess NADH. After 20 min in the dark, nitrite that formed in the reaction was measured colorimetrically by adding 640 uL 1% sulfanilamide in 3 M HCl. An equal volume of 0.02% N-(1-naftyl) ethylenediamine dihydrochloride was then added and incubated for 5 min. The sample was vigorously shaken and the absorbance was determined at 546 nm. For each series, blanks and a nitrite standard (20 uM KNO_2) were included. The activation state of NR was determined as a percentage ratio between NR activity measured in the presence of $MgCl_2$ (NR_{act}) and NR measured with EDTA (NR_{max}). Glutamine synthetase (GS) activity was determined as described by (Lacuesta et al., 1990) with minor modifications. Briefly, the reaction medium (final volume 105 uL) consisted of 100 mM HEPES-KOH (pH 7.8), 150 mM glutamate, 10 mM $MgCl_2$, 15 mM ATP, 10 mM NaOH, 10 mM hydroxylamine, and 2 mM EDTA. The reaction was started by adding 45 uL extract and terminated after 30 min by adding 150 uL 8%(w/v) trichloroacetic acid, 3.3% (w/v) $FeCl_3$, and 2 M HCl (Robredo et al., 2011).

For the measurement of root bleeding intensity, we used the method described by Li et al. (2012). In the afternoon, at 17:00, the wheat stem at 3–4 cm above the ground was cut, then use self-sealing bag containing absorbent cotton to attach the remaining lower stem and maintain contact between the absorbent cotton and the stem, next day at 17:00 in the afternoon, take the self-sealing bag back and weigh. The weight difference of the absorbent cotton is the root bleeding amount, and the root bleeding intensity was expressed as root bleeding amount one day.

The root samples were taken in the micro-plots. Three replications were sampled for NW and WW treatments at jointing. And six replications were sampled for NW, WW, SW and WSW treatments at anthesis and maturity. Roots of each micro-plot were collected and washed in four soil layers: 0–20, 20–40, 40–60 and 60–100 cm. Root length, root surface area and root volume were recorded digitally using a flatbed scanner (Epson Expression 1640XL-Pro, Seiko Epson Corp., Japan) and analyzed using WinRHIZO software (WinRHIZO PRO 2013, Reagent Instruments, Inc., Canada).

2.3. Calculation methods

N utilization efficiencies were calculated according to Duan et al. (2014):

N agronomic efficiency (NAE, kg^{-1}) = (grain yield at Nx − grain yield at N0)/N rate at Nx,

N recovery efficiency (NRE, %) = (plant N accumulation at Nx − plant N accumulation at N0)/N rate at Nx,

where Nx is N treatment and N0 is no N treatment.

The plant N accumulation and plant N accumulation rate were calculated according to Wang et al. (2015):

Plant N accumulation (kg ha^{-1}) = plant dry matter × plant N content,

Plant N accumulation rate (kg ha^{-1} d^{-1}) = plant N accumulation/growth duration.

The plant dry matter accumulation rate was calculated according to Takai et al. (2006):

Plant dry matter accumulation rate (kg ha^{-1} d^{-1}) = plant dry matter accumulation/growth duration.

The amount of soil inorganic N accumulation (N_{min}) was calculated according to Shi et al. (2012b):

N_{min} (kg ha^{-1}) = soil thickness × soil bulk density × soil inorganic N (NO_3^--N plus NH_4^+-N) content/10.

The soil N balances were calculated according to (Deng et al., 2014):

Apparent N surplus (ANS, kg ha^{-1}) = (initial N_{min} + N fertilization rate) − (residual N_{min} + plant N accumulation).

2.4. Statistical analysis

Statistical analyses were conducted using SPSS software (SPSS 17.0, SPSS, Inc., USA) for the standard analysis of variance (ANOVA). The attributes from different treatments were compared by the least significant difference (LSD) at 5% level. Graphics were drawn by using SigmaPlot software (SigmaPlot 10.0, Systat Software, Inc., USA).

3. Results

3.1. Grain yield, biomass, N accumulation, and N utilization efficiency

Night-warming treatments increased grain yield, biomass, N accumulation, NAE, and NRE of both cultivars, and the increases were greater for WSW than for WW and SW (Table 2). Night-warming treatments tended to reduce the number of spikes of both cultivars, but they were not significant compared to NW. WW and WSW significantly increased the number of grains of both cultivars, whereas SW only significantly increased the number of grains of Yangmai-13 during the 2015–2016 season. WW and WSW significantly increased the 1000-grains weight of both cultivars, whereas SW significantly increased the number of grains of Yangmai-13 during the 2014–2015 season and Yannong-19 during the 2015–2016 season. Grain yield, biomass, N accumulation, NAE, and NRE differed significantly by year (Table 3). Grain yield, biomass, N accumulation, NAE, and NRE differed significantly between the two cultivars, with a higher grain yield, biomass, NAE, and NRE in Yangmai-13 but a higher N accumulation in Yangnong-19.

3.2. Dry matter accumulation rate and N accumulation rate during different growth periods

WW and WSW increased dry matter accumulation, dry matter accumulation rate, N accumulation, and N accumulation rate of both cultivars from sowing to jointing (Table 4). The night-warming treatments increased dry matter accumulation, dry matter accumulation rate, N accumulation, and N accumulation rate of both cultivars from jointing to anthesis and from anthesis to maturity, and the increases

Table 2
Grain yield, biomass, N accumulation and N utilization efficiency affected by winter and spring night-warming in 2014–2016.

Cultivar	Treatment	Spikes ($\times 10^4$ ha^{-1})	Grain number	1000-Grains weight (g)	Grain yield (kg ha^{-1})	Biomass (kg ha^{-1})	N accumulation (kg ha^{-1})	NAE (kg kg^{-1})	NRE (%)
2014–2015									
Yangmai-13	NW	442.33a	45.63b	37.59c	7184.87c	15688.71c	171.83c	13.66c	36.04c
	WW	432.00a	49.30a	38.72a	7797.37b	16832.35ab	190.58ab	16.21ab	43.85ab
	SW	437.67a	46.85b	38.08b	7421.28c	16358.53b	180.66bc	14.65bc	39.72bc
	WSW	429.33a	50.85a	39.04a	8091.62a	17298.18a	197.92a	17.44a	46.91a
Yangnong-19	NW	489.67a	42.05c	35.62c	6794.44c	15087.54c	178.04c	12.90c	35.26c
	WW	483.33a	44.18ab	36.68ab	7400.61a	16130.96ab	196.20ab	15.42ab	42.82ab
	SW	487.33a	42.87bc	36.09bc	7074.37b	15683.07bc	187.24b	14.06bc	39.08bc
	WSW	476.67a	45.65a	37.06a	7659.89a	16643.99a	204.49a	16.50a	46.27a
2015–2016									
Yangmai-13	NW	416.33a	44.45c	38.27c	6780.83c	15438.83c	166.81c	13.72b	33.95b
	WW	405.67a	47.25a	39.36b	7681.60a	16742.43ab	188.88a	17.48a	43.15a
	SW	408.67a	46.05b	38.43c	7169.43b	16039.13bc	176.98b	15.34b	38.19b
	WSW	404.00a	48.15a	40.04a	7938.71a	17195.95a	195.76a	18.55a	46.01a
Yangnong-19	NW	508.33a	38.75b	34.13c	6411.62c	14632.92c	171.99d	12.97b	32.63b
	WW	494.33a	41.15a	35.61ab	7242.61b	16043.81a	194.46b	16.43a	41.99a
	SW	497.67a	39.35b	35.13b	6702.21c	15345.05b	181.02c	14.18b	36.39b
	WSW	492.33a	41.65a	35.84a	7544.23a	16572.05a	203.86a	17.69a	45.91a

NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. NAE and NRE refer to N agronomic efficiency and N recovery efficiency, respectively. Lower case letters refer to significant difference between treatments ($P < 0.05$).

were greater for WSW than for WW and SW. Furthermore, increases in the dry matter accumulation rate and N accumulation rate were higher from jointing to anthesis than from sowing to jointing and from anthesis to maturity. These results indicate that the night-warming treatments increased dry matter and N accumulation rates from jointing to anthesis, which improved the dry matter accumulation and N accumulation of winter wheat.

3.3. N concentration and N distribution in different organs at anthesis

The night-warming treatments increased the N concentration in leaves of both cultivars, and the increase was greater for WSW than for WW and SW (Table 5). The N concentrations in stems and spikes did not differ among the treatments. The night-warming treatments increased N distribution in stems, leaves, and spikes of both cultivars, but the increase was higher in leaves than in stems and spikes. The night-warming treatments increased the N distribution percentage in leaves but reduced it in stems. No difference in the N distribution percentage in spikes was observed among the treatments. These results indicate that the night-warming treatments promoted N distribution in leaves at anthesis, resulting in an increased N concentration in leaves.

3.4. Enzyme activity of nitrate reductase (NR) and glutamine synthetase (GS)

WW and WSW increased NR and GS activity in the leaves of both cultivars at the jointing stage (Fig. 3). The night-warming treatments increased NR and GS activity in the leaves of both cultivars at the booting, anthesis, and filling stages, and the increases were greater for WSW than for WW and SW. These results indicate that the night-warming treatments increased the N assimilation ability in leaves, which was conducive to N assimilation of winter wheat.

3.5. Root morphology and root bleeding intensity

WW and WSW increased root length, root surface area, and root volume in the 0–20 cm soil layer at the jointing stage but had no effects in the 20–40 and 40–60 cm soil layers (Fig. 4). The night-warming treatments increased root length, root surface area, and root volume in the 0–20, 20–40, and 40–60 cm soil layers at anthesis but had no effects in the 60–100 cm soil layer, and the increases were greater for WSW than for WW and SW. The night-warming treatments increased root length, root surface area, and root volume in the 0–20 and 20–40 cm soil layers at maturity but had no effects in the 40–60 and 60–100 cm soil layers, and the increases were greater for WSW than for WW and SW.

WW and WSW increased the root bleeding intensity of both cultivars at the jointing stage (Fig. 5). The night-warming treatments increased the root bleeding intensity of both cultivars at the booting, anthesis, and filling stages, and the increase was greater for WSW than for WW and SW. These results indicate that the night-warming treatments improved root morphology in the 0–60 cm soil layer and root activity, which was conducive to plant N uptake.

3.6. Soil urease and protease activity and soil net N mineralization rate

WW and WSW increased the soil urease and protease activity of both cultivars at the jointing stage (Fig. 6). The night-warming

Table 3
Variance analysis of grain yield, biomass, N accumulation and N utilization efficiency affected by year, cultivar and winter and spring night-warming.

Source	Spikes	Grain number	1000-Grains weight	Grain yield	Biomass	N accumulation	NAE	NRE
Year	ns	**	**	**	*	*	*	*
Cultivar	**	**	**	**	**	**	**	*
Treatment	ns	**	**	**	**	**	**	**
Y × C	**	**	**	ns	ns	ns	ns	ns
Y × T	ns	ns	ns	ns	ns	ns	ns	ns
C × T	ns	ns	ns	ns	ns	ns	ns	ns
Y × C × T	ns	ns	ns	ns	ns	ns	ns	ns

*and **indicate being significant at 0.05 and 0.01 levels, respectively. ns refer to no significant difference.

Table 4
Dry matter accumulation rate and N accumulation rate during different growth periods affected by winter and spring night-warming in 2014–2016.

Cultivar	Treatment	Dry matter accumulation (kg ha^{-1})			Dry matter accumulation rate (kg ha^{-1} d^{-1})			N accumulation (kg ha^{-1})			N accumulation rate (kg ha^{-1} d^{-1})		
		S-J	J-A	A-M	S-J	J-A	A-M	S-J	J-A	A-M	S-J	J-A	A-M
2014–2015													
Yangmai-13	NW	1824.80b	8163.43c	5700.48c	14.60b	189.85d	150.01c	53.17b	90.01c	28.65c	0.43b	2.09d	0.75c
	WW	1967.40a	8600.03ab	6264.92ab	15.74a	220.51b	152.80ab	57.83a	100.19ab	32.56ab	0.46a	2.57b	0.79ab
	SW	1824.80b	8476.23b	6057.50b	14.60b	206.74c	151.59bc	53.17b	96.78b	30.71bc	0.43b	2.36c	0.77bc
	WSW	1967.40a	8842.49a	6488.29a	15.74a	238.99a	154.60a	57.83a	105.71a	34.38a	0.46a	2.86a	0.82a
Yangnong-19	NW	1649.50b	7903.50c	5534.54c	13.20b	154.97d	158.13c	55.14b	91.88c	31.06d	0.44b	1.80d	0.89b
	WW	1807.10a	8231.30b	6092.56ab	14.46a	175.13b	160.33ab	61.12a	100.45b	34.63b	0.49a	2.14b	0.91ab
	SW	1649.50b	8139.80b	5893.77bc	13.20b	166.12c	159.29bc	55.14b	98.67b	33.43c	0.44b	2.01c	0.90ab
	WSW	1807.10a	8517.90a	6318.99a	14.46a	189.29a	162.03a	61.12a	107.35a	36.02a	0.49a	2.39a	0.92a
2015–2016													
Yangmai-13	NW	2037.50b	7857.60c	5543.73c	16.30b	191.65d	145.89c	57.14b	82.61c	27.06c	0.46b	2.02c	0.71c
	WW	2256.50a	8265.04ab	6220.89a	18.05a	217.50b	155.52a	64.24a	91.34b	33.29a	0.51a	2.40b	0.83a
	SW	2037.50b	8094.22bc	5907.41b	16.30b	207.54c	151.47b	57.14b	89.26b	30.58b	0.46b	2.29b	0.78b
	WSW	2256.50a	8520.39a	6419.06a	18.05a	236.68a	156.56a	64.24a	97.18a	34.35a	0.51a	2.70a	0.84a
Yangnong-19	NW	1805.75b	7592.41c	5234.76d	14.45b	158.18d	145.63c	59.18b	84.48d	28.32c	0.47b	1.76d	0.79c
	WW	2004.25a	8235.47ab	5804.09b	16.03a	187.17b	148.82b	67.06a	94.36b	33.04ab	0.54a	2.15b	0.85ab
	SW	1805.75b	7991.97b	5547.33c	14.45b	177.60c	145.98c	59.18b	91.07c	30.77bc	0.47b	2.02c	0.81bc
	WSW	2004.25a	8466.10a	6101.70a	16.03a	201.57a	152.56a	67.06a	101.64a	35.16a	0.54a	2.42a	0.88a

NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. S-J, J-A and A-M refer to the growth period of sowing to jointing, jointing to anthesis and anthesis to maturity, respectively. Lower case letters refer to significant difference between treatments ($P < 0.05$).

treatments increased the soil urease and protease activity of both cultivars at the booting, anthesis, and filling stages, and the increases were greater for WSW than for WW and SW.

WW and WSW increased the soil net N mineralization rate of both cultivars at the jointing stage (Fig. 7). The night-warming treatments increased the soil net N mineralization rate of both cultivars at the booting, anthesis, and filling stages, and the increase was greater for WSW than for WW and SW. These results indicate that the night-warming treatments increased soil urease and protease activity and the soil net N mineralization rate, which helped improve soil N supply.

3.7. Soil inorganic N content and apparent N surplus

WW and WSW decreased inorganic N content in the 0–40 cm soil layer of both cultivars at the jointing stage (Fig. 8). The night-warming treatments decreased inorganic N content in the 0–60 cm soil layer of both cultivars at the booting, anthesis, filling, and maturity stages, and the decreases were greater for WSW than for WW and SW. Soil inorganic N content at other stages did not differ among treatments.

The soil N balance was surplus from sowing to jointing, and WW and WSW slightly decreased the soil N surplus, but the difference was not significant (Table 6). The soil N balance was surplus from jointing to anthesis. The night-warming treatments significantly decreased the soil N surplus, except for SW in Yangmai-13 in 2014–2015, and the

Table 5
N concentration and N distribution in different organs at anthesis affected by winter and spring night-warming in 2014–2016.

Cultivar	Treatment	N concentration (g kg^{-1} DW)			N distribution (mg culm^{-1})			N distribution percentage (%)		
		Stem	Leaf	Spike	Stem	Leaf	Spike	Stem	Leaf	Spike
2014–2015										
Yangmai-13	NW	8.04a	33.95d	19.11a	12.26c	13.90c	6.22c	37.87a	42.93c	19.20a
	WW	8.10a	36.25b	19.03a	13.18ab	16.41a	7.00ab	36.03c	44.85a	19.12a
	SW	7.97a	35.24c	18.94a	12.58bc	15.09b	6.59bc	36.71b	44.06b	19.23a
	WSW	8.21a	36.70a	18.83a	13.67a	17.22a	7.20a	35.88c	45.21a	18.90a
Yangnong-19	NW	8.68a	36.62d	21.85a	11.67b	12.67d	5.69c	38.86a	42.20b	18.94ab
	WW	8.86a	38.96b	21.49a	12.50a	14.70b	6.25b	37.37b	43.95a	18.68b
	SW	8.75a	38.09c	21.86a	12.09ab	13.48c	6.01bc	38.28a	42.70b	19.02ab
	WSW	8.86a	39.53a	21.19a	12.82a	15.66a	6.87a	36.26c	44.31a	19.43a
2015–2016										
Yangmai-13	NW	7.94a	32.87d	18.74a	12.50b	14.06d	7.02b	37.24a	41.86d	20.90a
	WW	7.96a	35.88b	18.66a	13.51a	16.92b	7.92a	35.24bc	44.11b	20.65a
	SW	7.94a	34.47c	18.63a	12.98b	15.45c	7.40b	36.23ab	43.13c	20.64a
	WSW	8.02a	36.22a	18.52a	13.91a	17.94a	8.11a	34.81c	44.91a	20.28a
Yangnong-19	NW	8.60a	37.37c	20.08a	10.69c	11.73d	5.87c	37.79a	41.47c	20.74a
	WW	8.49a	39.62ab	19.53a	11.49ab	14.09b	7.07a	35.19c	43.15b	21.66a
	SW	8.49a	38.11bc	19.54a	11.10bc	12.67c	6.42b	36.76b	41.97c	21.27a
	WSW	8.58a	40.15a	19.59a	11.80a	15.12a	7.36a	34.41d	44.11a	21.48a

NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. Lower case letters refer to significant difference between treatments ($P < 0.05$).

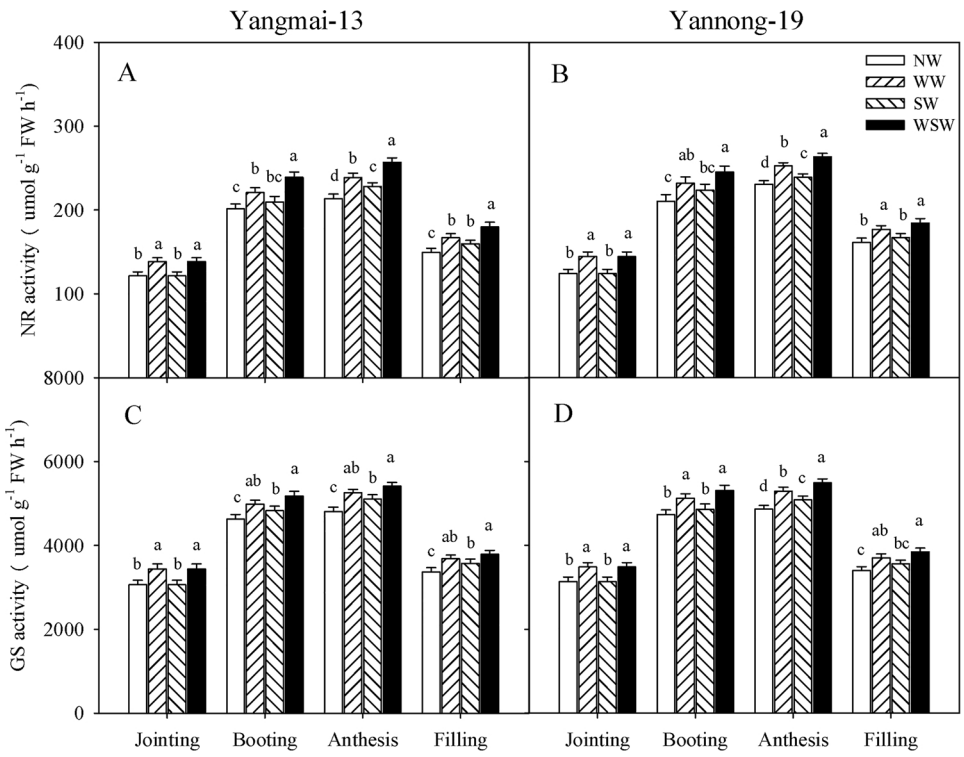

Fig. 3. Enzyme activity of nitrate reductase (NR) and glutamine synthetase (GS) affected by winter and spring night-warming of Yangmai-13 (A, C) and Yannong-19 (B, D) in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. The top expanded leaves at jointing and the flag leaves at booting, anthesis and filling were sampled. Filling refers to 14 days after anthesis. Lower case letters refer to significant difference between treatments ($P < 0.05$). Whiskers on the top of the bars indicate standard error.

decrease was greater for WSW than for WW and SW. The soil N balance was deficit from anthesis to maturity, and the night-warming treatments slightly increased the soil N deficit, but the difference was not significant. The soil N balance was surplus from sowing to maturity, and the night-warming treatments decreased the soil N surplus. The decrease was greater for WSW than for WW and SW. These results indicate that the night-warming treatments decreased the soil N surplus from jointing to anthesis to decrease soil N surplus from sowing to maturity, which was caused by highly improved plant N uptake capacity, resulting in reduced leaching loss of N fertilizer.

4. Discussion

Winter and spring night-warming increased the grain yield and N utilization efficiency of winter wheat and reduced the soil apparent N surplus (Tables 2 and 6). Grain yield and N accumulation differed

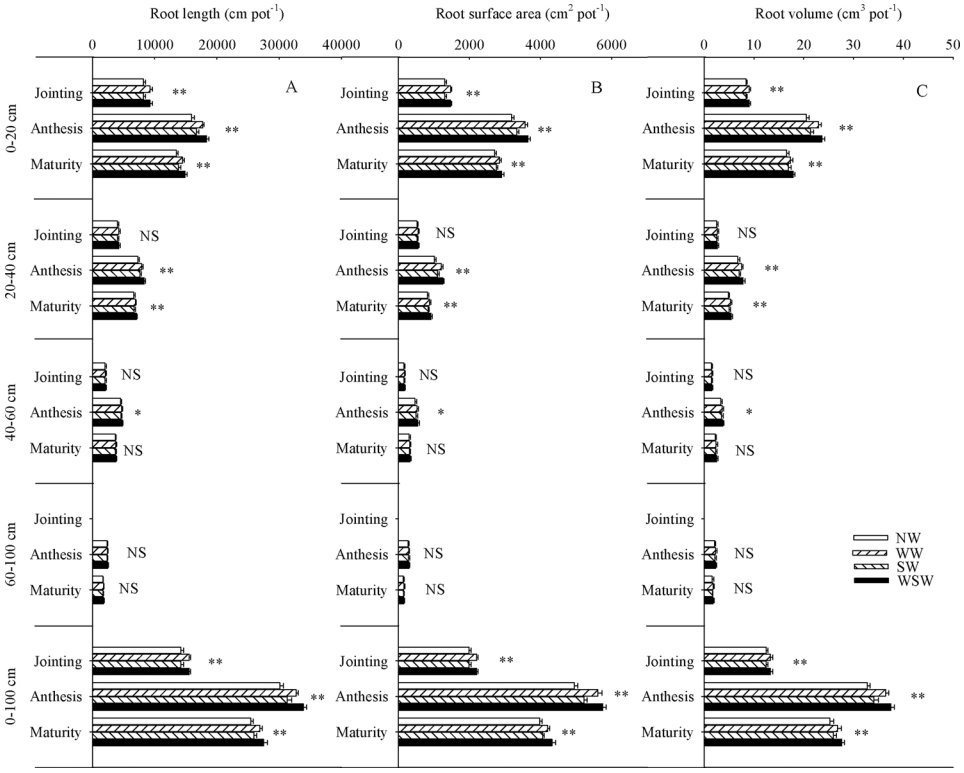

Fig. 4. Root length (A), root surface area (B) and root volume (C) in 0–100 cm soil layer affected by winter and spring night-warming of Yangmai-13 in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. ** and * indicate significant difference between treatments and control at the 0.01 and 0.05 level and NS indicate no significant difference.

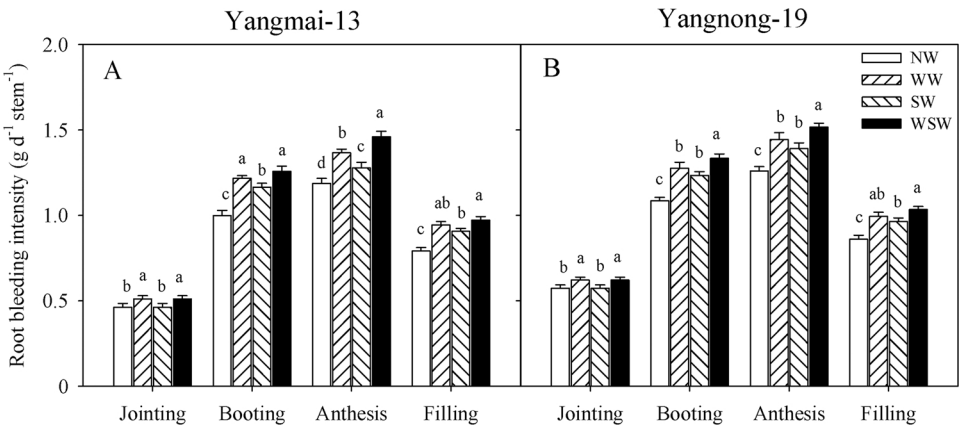

Fig. 5. Root bleeding intensity of Yangmai-13 (A) and Yannong-19 (B) affected by winter and spring night-warming in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. Filling refers to 14 days after anthesis. Whiskers on the top of the bars indicate standard error.

significantly between the two cultivars (Tables 2 and 3), with a higher grain yield in Yangmai-13 than in Yannong-19 but a higher N accumulation in Yangnong-19 than in Yangmai-13. These results indicate that Yangmai-13 had a higher N uptake ability, whereas Yangnong-19 had a higher photosynthetic ability to enhance grain yield (Fan et al., 2015; Fan et al., 2017). Increases in wheat NAE are mainly due to increases in grain yield. Some studies have reported that warming reduces the grain yield of winter wheat in China because of large declines in biomass production (You et al., 2009; Liu et al., 2013). However, those studies mainly focused on the entire growth period of wheat and did not consider asymmetric warming. In this study, the night-warming treatments increased the number of grains and 1000-grains weight of both cultivars, resulting in an increased grain yield of winter wheat, and the increase was greater for WSW than for WW and SW (Table 2). Furthermore, the night-warming treatments increased the dry matter accumulation rate from sowing to jointing, from jointing to anthesis, and from anthesis to maturity, particularly from jointing to anthesis (Table 4), resulting in increased biomass, which helped improve grain yield. Leaf N concentration is one of the most important plant N variables determining photosynthetic carbon fixation and plant productivity (An et al., 2005). Some studies have reported that warming increases or decreases leaf N concentration (Sardans et al., 2008b; Volder et al., 2015; White-monsant et al., 2017). However, most of these studies were carried out in grassland or forest ecosystems, and very few studies have been performed on winter wheat. In this study, the night-warming treatments increased the N distribution and the N distribution percentage in leaves at anthesis (Table 5), resulting in an increased N concentration in leaves, which helped increase post-anthesis photosynthetic ability and improve the grain yield of winter wheat (Fan et al., 2015).

Increases in wheat NRE are mainly due to an increase in N accumulation. Zhang et al. (2013) reported that night-warming increases wheat N accumulation by 17% to 43% at the jointing, anthesis, and maturity stages. Similarly, in this study, the night-warming treatments increased the N accumulation rate from sowing to jointing, from jointing to anthesis, and from anthesis to maturity, particularly from jointing to anthesis, resulting in increased N accumulation at maturity (Tables 2 and 4). Root bleeding intensity is the amount of sap-flow per unit time, which can be used as an index of root activity (Zhang et al., 2012). Plant N uptake ability is closely related to root morphology and activity (Kamh et al., 2005; Foulkes et al., 2009; Dai et al., 2013). Björk et al. (2007) reported that long-term warming increases specific root length and specific root area in two dry tundra plant communities. Tian et al. (2014) reported that experimental warming increases root dry

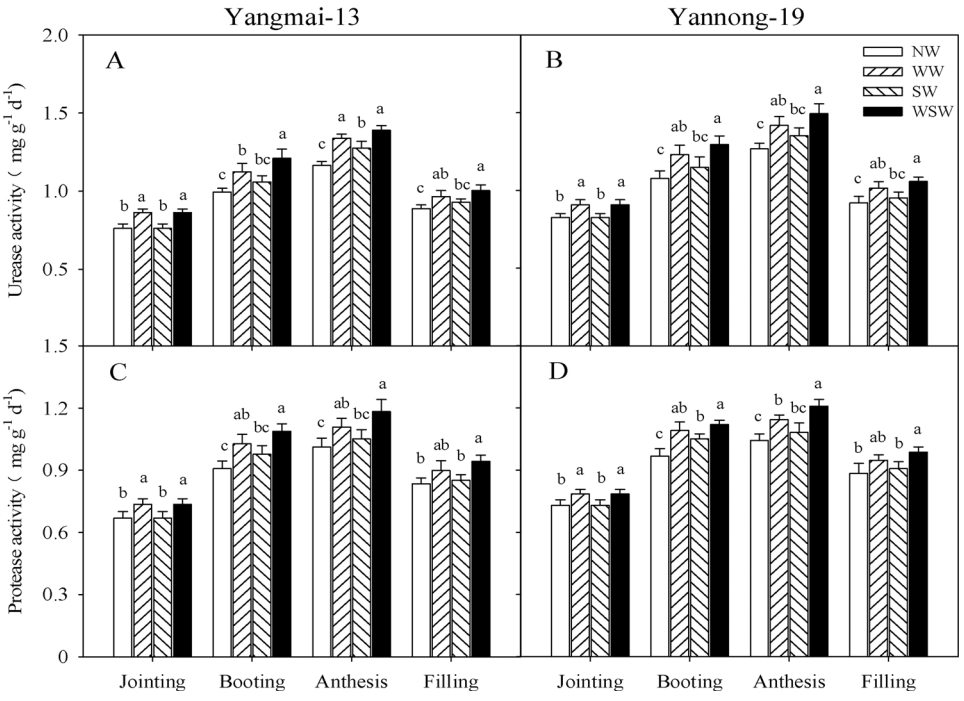

Fig. 6. Urease and protease activity in the 0–20 cm soil layer affected by winter and spring night-warming in Yangmai-13 (A, C) and Yannong-19 (B, D) in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. Filling refers to 14 days after anthesis. Whiskers on the top of the bars indicate standard error.

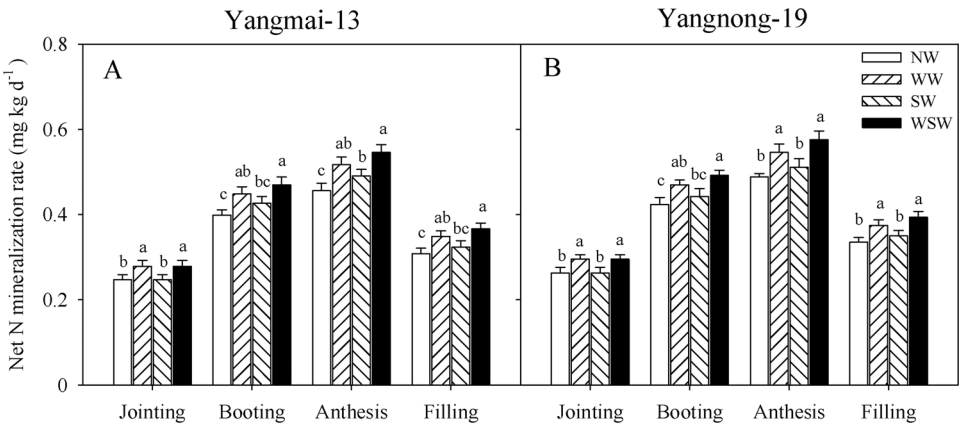

Fig. 7. Net N mineralization rate in the 0–20 cm soil layer affected by winter and spring night-warming of Yangmai-13 (A) and Yannong-19 (B) in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. Filling refers to 14 days after anthesis. Whiskers on the top of the bars indicate standard error.

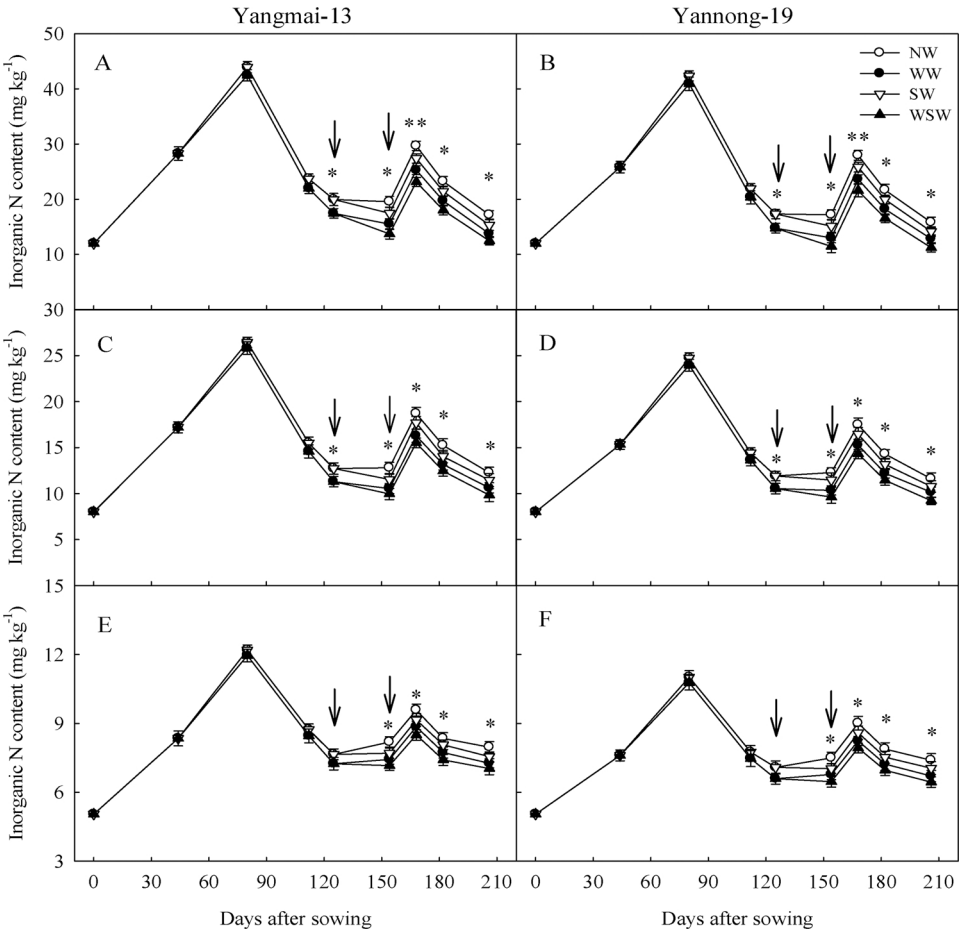

Fig. 8. Inorganic N content in 0–60 cm soil layer affected by winter and spring night-warming in Yangmai-13 (A, C, E) and Yannong-19 (B, D, F) in 2015–2016. NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. ↓ represents N topdressing time. A and B represent 0–20 cm soil layer, C and D represent 20–40 cm soil layer, E and F represent 40–60 cm soil layer. Whiskers on the top of the bars indicate standard error. ** and * indicate significant difference between treatments and control at the 0.01 and 0.05 level.

biomass and activity of winter wheat at anthesis, which helps improve plant N uptake. In this study, the night-warming treatments increased root length, root surface area, and root volume in the 0–60 cm soil layer (Fig. 4), resulting in improved root morphology that was conducive to plant N uptake. Moreover, the night-warming treatments increased root bleeding intensity at the jointing, booting, anthesis, and filling stages (Fig. 5), which may also have contributed to higher plant N uptake. Furthermore, the increases in root length, surface area, and volume in the 0–20 cm soil layer were greater than those in the 20–40 and 40–60 cm soil layers, which indicates that the night-warming treatments mainly improved root morphology in the 0–20 cm soil layer.

NH_4^+-N is easily converted into NO_3^--N by nitrification, and NO_3^--N is the main nitrogen source for land crops (Asseng et al., 2001). NO_3^--N absorbed by wheat is reduced to nitrite in the cytosol by NR, and ammonium is then converted into glutamine and glutamate in the plastid/chloroplast by GS system (Lacuesta et al., 1990; Singh et al., 2014). These reactions present a bottleneck to growth and yield and can potentially affect NUE (Thomsen et al., 2014). Jauregui et al. (2015) reported that leaf photorespiration and dark respiration are inhibited at elevated temperatures (+4 °C), resulting in decreased NR and GS activity in wheat leaves. However, in this study, the night-warming treatments increased NR and GS activity in leaves at the jointing, booting, anthesis, and filling stages (Fig. 3). This may be because the temperature increase was too great, and in this study the night-warming treatments moderately increased the night temperature during winter and spring (Fig. 2). The increased NR and GS activity in leaves improved N assimilation ability, resulting in improved wheat N accumulation.

Table 6
Apparent N surplus (kg ha^{-1}) in 0–60 cm soil layer affected by winter and spring night-warming in 2014–2016.

Cultivar	Treatment	S-J		J-A		A-M		S-M	
		2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016
Yangmai-13	NW	28.09a	31.82a	20.04a	16.97a	0.43a	-1.67a	48.56a	47.12a
	WW	25.80a	29.68a	14.50b	10.90b	-1.33a	-2.08a	38.97b	38.50b
	SW	28.09a	31.82a	17.68a	13.54b	-0.21a	-1.71a	45.57a	43.64a
	WSW	25.80a	29.68a	12.11b	7.67c	-2.88a	-3.41a	35.03b	33.94c
Yangnong-19	NW	24.52a	27.74a	16.26a	12.95a	-2.08a	-4.51a	38.71a	36.18a
	WW	21.88a	25.43a	10.86b	5.92bc	-4.30a	-5.85a	28.44bc	25.49b
	SW	24.52a	27.74a	13.33b	9.02b	-3.29a	-5.16a	34.57ab	31.61a
	WSW	21.88a	25.43a	7.35c	2.61c	-5.11a	-6.76a	24.12c	21.27b

NW, WW, SW and WSW refer to no warming control, winter night-warming, spring night-warming and winter + spring night-warming, respectively. S-J, J-A, A-M and S-M refer to the growth period of sowing to jointing, jointing to anthesis, anthesis to maturity and sowing to maturity, respectively. Lower case letters refer to significant difference between treatments ($P < 0.05$).

Temperature is generally thought to be an important factor controlling soil enzyme activity (Baldrian et al., 2013). Several studies have revealed that environmental warming can induce higher soil enzyme activity (Kang and Freeman, 1999; Allison and Treseder, 2008; Xu et al., 2010). Moreover, Bai et al. (2013) reported that warming increases the soil net N mineralization rate to increase soil inorganic N content, which helps increase plant N uptake. In this study, the night-warming treatments increased urease and protease activity and the net N mineralization rate in the 0–20 cm soil layer at the jointing, booting, anthesis, and filling stages (Figs. 6 and 7), which helped improve soil N supply, resulting in improved plant N uptake. Wang et al. (2014) reported that both short-term and long-term warming significantly increases soil urease activity and NH_4^+-N content in an alpine meadow ecosystem, which likely favors plant growth and productivity. However, in this study, the increased soil urease and protease activity as well as soil net N mineralisation rate were not accompanied by an increase in soil inorganic N content (Fig. 8). This may be because the experimental site was different and the night-warming treatments increased plant N accumulation (Table 4). Soil inorganic N is easily absorbed by plants and represents the capacity of soil N supply: Higher inorganic N content in the soil is beneficial for plant N uptake but carries a risk of leaching from the soil profile (Liu et al., 2003). The night-warming treatments decreased inorganic N content in the 0–60 cm soil layer, particularly at the maturity stage (Fig. 8), which helped reduce the risk for N leaching loss after harvest (Patil et al., 2010).

The soil N balance is an important index for evaluating whether soil N supply can satisfy crop N demand (Cassman et al., 2002; Guarda et al., 2004). The soil N surplus and deficit are linked to growth-stage features due to differences in N supply and crop N demand (Shi et al., 2012b). In this study, the soil N balance was surplus from sowing to jointing and from jointing to anthesis, which indicates that soil N supply fully met the N demand of wheat plants during these periods; the soil N balance was deficit from anthesis to maturity, which may be due to the large amount of N uptake before anthesis (Table 6). Several studies have reported that surplus N can be lost by NO_3^--N leaching and pollute the environment (Öborn et al., 2003; Sieling and Kage, 2006). In this study, the night-warming treatments decreased the soil N surplus from sowing to maturity, which was caused by enhanced plant N uptake capacity, resulting in reduced leaching loss of surplus N (Tables 4 and 6). Furthermore, the main period of N surplus is from sowing to jointing; therefore, decreasing basal N and increasing top-dressing N is likely to be a good strategy for reducing soil N over-supply during the early stage and alleviating soil N deficits during the late stage to coordinate the soil N supply and N demand of winter wheat under future climate warming conditions.

5. Conclusion

Winter and spring night-warming increased N uptake and utilization of winter wheat, and WSW (warming by 1.53–1.60 °C) had greater advantages in this respect than WW (warming by 1.47–1.53 °C) and SW (warming by 1.68–1.77 °C). Winter and spring night-warming improved root growth and soil N supply, which resulted in increased plant N uptake. Moreover, winter and spring night-warming improved plant N assimilation ability and N distribution in leaves at anthesis. The soil apparent N surplus was decreased under winter and spring night-warming, which was caused by highly improved plant N uptake capacity, resulting in reduced loss of N fertiliser. The findings of this study should be considered for crop modelling of wheat to improve NUE under future climate change.

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